A Twist on Potassium Channel Gating

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The selectivity filter of potassium channels, the narrowest segment of the pore, permits only potassium ions to diffuse through the pore. Clarke et al. (2010) now present 11 structures of an inwardly rectifying potassium channel, providing evidence that the selectivity filter functions in channel gating and conformational changes in the cytoplasmic domains correlate with pore opening.

Potassium channels regulate resting membrane potential of many cell types, including neurons, cardiac myocytes, and pancreatic islet cells. They are separated into families on the basis of regulatory domains that control the opening and closing, or gating, of the ion-conducting pore. For example, voltage-gated channels possess a voltage-sensing domain, and inwardly rectifying potassium (Kir) channels, which pass potassium ions more easily into than out of the cell, contain a cytoplasmic regulatory domain. In Kir channels, this domain interacts with signaling molecules (Jan and Jan, 1997), but it also creates the cytoplasmic portion of the ion pore (Figure 1) (Nishida and MacKinnon 2002). In all potassium channels, the transmembrane portion of the pore is lined with a selectivity filter that allows only potassium ions to pass through the channel. It has been a long-standing question how gating signals in the cytoplasmic domain are transmitted to the transmembrane portion of the pore. In this issue of *Cell*, Clarke et al. (2010) take great strides toward resolving this issue by presenting 11 snapshots of an inwardly rectifying potassium channel from bacteria, KirBac, in various states of the gating process. These structures indicate that the selectivity filter is involved in gating the KirBac channel and that global conformational changes in the channel propagate the gating signal from the cytoplasmic domains to the transmembrane domains.

Seven years ago, the X-ray crystal structure of KirBac provided the first portrait of a full-length potassium channel with both transmembrane and cyto-

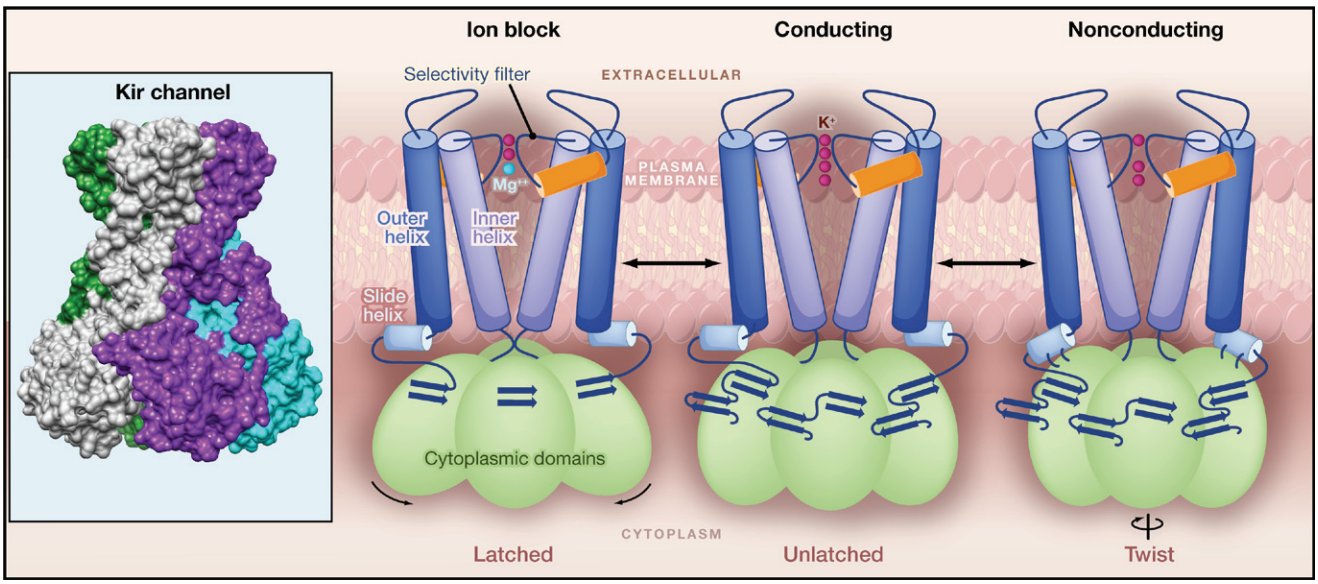

Figure 1. Kir Channel Cytoplasmic Domains Communicate with the Selectivity Filter
(Left) Inwardly rectifying potassium channels, such as the bacterial KirBac (PDB: 1P7B), contain four subunits (green, gray, purple, and cyan), which twist together to form an ion permeation pore created by both the transmembrane and cytoplasmic domains.
(Right) To see the ion permeation pore, this panel shows the transmembrane (blue and orange) and cytoplasmic (green) domains of KirBac for only two and four subunits, respectively. Clarke et al. (2010) now demonstrate that conformational changes in the cytoplasmic domains correlate with the ion configuration in the selectivity filter and the diameter of the cytoplasmic portion of the pore. In the inactive state, the cytoplasmic domains have four latched interfaces and a divalent cation prevents conductance through the pore. When these interfaces unlatch, or form an intermolecular β sheet between neighboring subunits, the selectivity filter is then in a state that actively conducts ions. In contrast, a global rotation or twist of the cytoplasmic domains alters the conformation of the slide helix and correlates with a configuration in the selectivity filter that does not conduct ions.

plasmic domains (Kuo et al., 2003). Like other potassium channels (Doyle et al., 1998), the transmembrane pore of KirBac is created by four subunits, each composed of an inner helix, an outer helix, and a pore loop between these helices (Figure 1). The four pore loops circle around the channel axis to form the highly conserved selectivity filter. Near the cytoplasmic surface of the membrane is another bottleneck in the pore, called the helix-bundle crossing. This constriction is thought to govern channel gating by converting between multiple open and closed configurations.

Although the transmembrane pores in the new structures presented by Clarke et al. exhibit little variation from previous potassium channel structures, the cytoplasmic regulatory domains display conformations unseen in previous structures (Kuo et al., 2003; Nishida and MacKinnon, 2002). Eight of the 11 structures presented in the new study possess a 23° rotation of the cytoplasmic domain relative to the transmembrane pore (Figure 1, right). This twist configuration is absent in the other three structures and is associated with movement of the slide helix, a motif near the bundle crossing that is commonly involved in gating potassium channels (Figure 1). An interaction between the slide helix and its neighboring subunit stabilizes the twisted state and creates a structural link from cytoplasmic regulatory domains to the transmembrane domains, where the selectivity filter resides.

Although the twist conformation does not significantly alter the backbone residues of transmembrane helices (at the moderate resolution of 2.6 Å to 4.6 Å), remarkably, the ion configurations within the selectivity filter correlate with the twisting of the cytoplasmic domain. This suggests a global conformational relay that transduces structural rearrangements of the cytoplasmic domain to the selectivity filter via the slide helix. Previous studies defined five cation-binding sites, S0–S4, within the selectivity filter. In structures with the twist conformation, three ions occupy the selectivity filter at sites S1, S3, and S4, which corresponds to a state in which ion conduction is predicted to be stalled. In structures with the nontwist conformation, the selectivity filter contains either three (S1, S2, S3 or S1, S2, S4, with S3 or S4 being occupied by Mg^{2+} or Ca^{2+}) or four (S1–S4) ions, but, most importantly, K^+ is always in the S2 site. Interestingly, the presence of K^+ in the S2 site is thought to inhibit the transition from an actively conducting to a nonconducting conformation of the selective filter (Bernèche and Roux, 2005). Therefore, the selectivity filter associated with the nontwist conformation is likely in a state that conducts K^+ atoms (Figure 1, middle).

Clarke et al. find that in addition to rotating, cytoplasmic domains of the KirBac channel also undergo structural rearrangements that create two distinct types of interfaces, called latched and unlatched (Figure 1). Compared to the latched interface, the unlatched one displays more extensive interactions between subunits; the N-terminal domain interweaves with its neighboring subunit by forming an intermolecular β sheet between the two cytoplasmic domains. Structurally, unlatching of the subunits dilates the cytoplasmic portion of the pore like the iris dilates the pupil of an eye. Importantly, the unlatching also correlates with the ion conduction status of the selectivity filter. Analysis by the authors indicates that the selectivity filter is in the conducting conformation when all four interfaces are unlatched (Figure 1, middle).

The outward current of inwardly rectifying Kir channel is blocked by intracellular factors such as Mg^{2+}, Ca^{2+}, and polyamines (Lu, 2004). Clarke et al. now identify two types of binding pockets on KirBac for the polyamine spermine. One site is located at the latched interface between subunits. The second site, which is present only in structures with unlatched interfaces, sits deep in the central ion permeation pore at the bundle crossing. This second spermine site is consistent with physiological data showing that negatively charged residues at this location in strongly inwardly rectifying potassium channels interact with positively charged polyamines. Thus, unlatching subunits of the channel not only modifies the opening of the cytoplasmic pore and the conductive status of the selectivity filter (Figure 1), but also releases the spermine from its binding pocket at the subunit interface. This polyamine subsequently plugs the conducting pore and prevents potassium ions from exiting the cell.

The C linker, which is adjacent to the helix-bundle crossing, connects the transmembrane inner helix to the cytoplasmic domain (Figure 1). The C linker is known to interact with negatively charged phospholipid head groups, in particular phosphatidyl inositide, an essential regulator of potassium ion channels (Suh and Hille, 2008). Using improved data processing algorithms, Clarke et al. (2010) reanalyze previous structures of KirBac and can now assign electron density near the C linker to a phospholipid head group. This lipid further narrows the pore opening at the helix-bundle crossing, suggesting a direct role for this lipid binding site in channel gating. However, given the absence of a lipid bilayer in the crystal environment, the physiological relevance of this constriction is still unknown.

Overall, the new study by Clarke et al. demonstrates that the selectivity filter forms distinct conductive configurations without markedly altering the backbone of residues lining the selectivity filter; instead, changes in the conductivity of the selectivity filter correlate with remote conformational changes of the cytoplasmic domains. Thus, both transmembrane and cytoplasmic domains work together as a network to relay conformational changes across the entire channel.

Ever since the discovery of a gating mechanism in Shaker channels that depends on the extracellular opening of the channel, referred to as C-type inactivation, the potential involvement of the selectivity filter has been actively discussed (Hoshi et al., 1991). The structural data presented by Clarke and colleagues now suggest that the selectivity filter plays a role in gating by directly responding to global conformational changes of the channel. Future studies with higher-resolution structures combined with molecular simulation and electrophysiological analysis will provide further insight into how these changes are transmitted and how this mechanism is implemented in other families of potassium channels.

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Autophagy Shows Its Animal Side

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Most autophagy genes have been discovered in the single-celled yeast *Saccharomyces cerevisiae*, and little is known about autophagy genes that are specific to multicellular animals. In this issue, Tian et al. (2010) now identify four new autophagy genes: one specific to the nematode *Caenorhabditis elegans* and three conserved from worms to mammals.

Autophagy, or "self-eating," is a catabolic process that degrades and recycles cytoplasmic contents. Pioneering studies in the single-celled yeast *Saccharomyces cerevisiae* identified a suite of autophagy (*Atg*) genes required for survival during starvation (Mizushima, 2007). Although many of these genes are functionally conserved from yeast to mammals, autophagy is probably more complex in multicellular animals and most likely requires factors that are absent in yeast. For example, animal tissues maintain homeostasis when nutrients are locally restricted by trading off metabolic and catabolic processes, and this may be one reason that cancer cells with altered metabolism display elevated levels of autophagy (Mathew et al., 2007). However, little is known about autophagy machinery specific to animals. Now in a tour de force study, Tian et al. (2010) identify four previously uncharacterized genes specifically required for autophagy in multicellular animals and establish *Caenorhabditis elegans* as one of the premier genetic models for uncovering new autophagy genes in animals.

During autophagy, cytoplasmic contents, such as proteins and organelles, are engulfed by a double-membrane autophagosome (Figure 1), which then fuses with lysosomes to form autolysosomes. Here hydrolase enzymes degrade the cargo, and the products are subsequently released into the cytosol for reuse (Mizushima, 2007). Besides recycling cytoplasmic material during periods of starvation or stress, autophagy (also called macroautophagy) clears protein aggregates, eliminates pathogens, and influences cell death. Moreover, in many organisms, autophagy defects are associated with decreased life span, neurodegeneration, and tumor progression (Mizushima et al., 2008).

In worms (*C. elegans*), flies, and mammals, autophagy is also important during development (Meléndez and Neufeld, 2008). In *C. elegans*, germ cells contain aggregates of protein and RNA known as P granules, which are absent in somatic cells. A previous study demonstrated that autophagy is required for clearing the aggregate-prone components of P granules from somatic cells in developing *C. elegans* embryos (Zhang et al., 2009), and defects in autophagy lead to the aberrant accumulation of aggregates of P granule proteins in somatic cells.

Now Tian et al. (2010) use the persistence of P granule proteins in somatic cells to find mutant *C. elegans* embryos with defects in autophagy. From the ~160 mutants identified, the authors isolated four new genes, named *epg-2*, *-3*, *-4*, and *-5* (*e*ctopic *P*GL *g*ranules), which do not map to known autophagy genes. The coiled-coil protein, *epg-2*, mediates recognition of cargo (e.g., aggregates of P granule proteins) for delivery to autophagosomes and appears to be specific to nematodes. The other three genes, *epg-3*, *-4*, and *-5*, are also required for starvation-induced autophagy. They are conserved genetically from worms to mammals and appear to lack homologs in yeast.

In addition, the authors isolated numerous new mutations in genes homologous to yeast autophagy genes, which validate and strengthen the results of the study. Not only do these new mutations provide a valuable resource for probing the structure and function of autophagy proteins, but they also establish *C. elegans* as a preeminent system for studying the role and regulation of autophagy in multicellular animals.